page 3.) Applicants teachings are not limited to this, however. The present application also provides detailed instructions for the making of *purified* preparations of the claimed compositions (see, *e.g.*, the paragraph in Example 3 on page 71 at lines 14-30.)

II. The Specification Teaches One Skilled in the Art How to Use the Claimed Compositions.

Applicants have provided extensive teachings enabling one skilled in the relevant art to make and use enzymes comprising a heterologous functional domain, and enabling one skilled in the art to test any such enzyme for improved background specificity.

The Examiner acknowledges that Applicants have provided rapid screening assays for measuring enzyme activities in crude cell lysates. However, Applicants' teachings are not limited to this. Applicants provide detailed description of how to use both crude lysates (e.g., from cells expressing these enzymes) and purified enzymes in assays to measure cleavage activity on a variety of substrate cleavage structures. For example, Example 1 includes detailed protocols for conducting cleavage assays, including examples of substrates, reaction conditions, and methods for detecting the products of the cleavage reactions. For example, as specificed in Example 1 at page 57, lines 21-22, approximately 20 ng of each of several purified mutant enzymes were used in the screens that generated the data presented in Tables 2-7 and in Figures 12, 14, 15, 19 and 25. In the paragraph immediately preceding, at page 57 lines 14-20, additional teachings of enzyme testing conditions are provided.

The Examiner has not alleged that the specification does not enable one of skill in the relevant art to make and/or use enzymes comprising heterologous functional domains. Rather, the Examiner's sole ground for alleging non-enablement of the claimed invention is his allegation that Applicants have not shown *possession* of enzymes providing "improved background specificity". However, this is not correct. Applicants have provided numerous examples of newly created enzymes having improved background specificity, as described below.

III. The Specification Conveys to One Skilled in the Art That The Inventors Were In Possession of the Claimed Invention At The Time the Application Was Filed.

The Examiner admits that the specification provides support for the "improved background specificity" of the claimed compositions (Office Action mailed October 7, 2002, page 2.) As described in the Summary of the Invention (*e.g.*, on page 3, line 24 through page 4, line 3), improved background specificity relates to an increased difference between the detectable amount of cleavage of a specific structure (*e.g.*, IdT and IrT1 structures described on page 55 of

the specification, which may be used to measure activity of any enzyme on DNA and RNA invasive cleavage structures, respectively), and the detectable amount of cleavage of any alternative structures such as might contribute to undesirable background in a particular assay (*e.g.*, the X and HP substrates depicted in Fig. 22 A and 22B). Improvements in the enzymes of the invention are generally determined by comparison of the function(s) of a test enzyme to the function(s) of a reference enzyme.

As further described on pages 3-4 of the present application, improved background specificity of the claimed enzymes may arise from a number of combinations of changes in the rate of cleavage of either or both specific and alternative structures. Improvement may arise, for example, from an increase in activity on a specific structure combined with a lesser increase, no increase or a decrease in activity on one or more alternative structures. Similarly, it may arise from a decrease in activity on one or more alternative structures combined with a lesser decrease or no decrease in activity on a specific structure. Applicants have provided examples of enzymes showing improved background specificity by EACH of these combinations of activity changes.

Comparison of the turnover rates between a number of the test nucleases and reference nucleases are provided in Tables 2-7. The activity of these enzymes on specific structures is shown both as a cleavage rate (i.e., turnover rate) and as an indicated percentage of the activity of a reference nuclease. The comparison of the enzymes being screened or tested to reference or control enzymes is discussed in Example 1, at page 52 at lines 3-7. One skilled in the art, reading the Specification, would understand the columns indicated as "%Tth" and "%Taq4M" as referring to the activity of the test enzyme indicated as a percentage of the activity of Tth enzyme or the Taq4M enzyme, respectively, tested under the same conditions. Where the percentage of a reference activity for test enzyme on a particular substrate has not been provided explicitly (e.g., for the HP and X structures), it can be readily calculated from the turnover rates provided in these tables for the test and reference enzymes on these substrates. These tables show that numerous enzymes having improved background specificity were demonstrated by and possessed by the inventors at the time of filing.

Examples of the claimed compositions possessed by the inventors include (but are in no way limited to) the following:

1. **Taq 4M L109F/A110T (Table 3):** When L109F and A110T mutations were added to the Taq 4M variant, the activity on the IrT1 test substrate was modestly decreased to 92% of the activity the Taq 4M enzyme, while the activity on the X structure was reduced to 68% of the X structure activity of Taq 4M. In addition, the activity on the HP structure

was reduced to only 29% of that of the Taq 4M enzyme. This improvement comprises reductions in activity on all of these structures, but the reduction of cleavage on the alternative structures is greater, thus background specificity is improved.

2. **Tth DN RX HT H786A/G506K/Q509K (AKK)(Table 2):** When H786A, G506K and Q509K mutations were added to the Tth DN RX HT variant, the activity on the IrT1 test substrate was increased to 179% of the activity the Tth enzyme on this substrate, while the activity on the HP structure was increased by a lessor amount, to 150% of the HP structure activity of the Tth enzyme. This improvement comprises increases in activity on both of these structures, but the increase of cleavage on the alternative structures is smaller, thus background specificity is improved.

3. **Taq 4M R587A (Table 2)**: When an R587A mutation was added to the Taq 4M variant, the activity on the IrT1 test substrate was increased to 118% of the activity the Taq 4M enzyme, while the activity on the X structure was reduced to 23% of the X structure activity of Taq 4M.. This improvement in background specificity comprises increased cleavage of a specific structure and decreased cleavage of the alternative X structure, thus background specificity is improved.

4. **Tth DN RX HT H786A/G203R (Table 5):** When the H786A and G203R mutations were added to the Tth DN RX HT variant, the activity on the IrT1 test substrate was increased to 180% of the activity the Tth enzyme on this substrate, while the activity on the X structure was reduced modestly to 97% of that of the Tth enzyme. This alone constitutes an improvement in background specificity. In addition, the activity on the HP structure was reduced to 55% of the HP structure activity of the Tth enzyme, which is an additional improvement in background specificity.

As detailed above, the subject matter of Claims 1-8, 10, 12-15, 18, 19 and 50-57 is described in the specification is such a way as to enable one skilled in the art to make and use the invention. Specifically, the Application provides extensive teachings on how to make and use enzymes having a heterologous functional domain providing improved background specificity. Further demonstrating that these teachings are enabling, Applicants provide data showing possession at the time of filing of numerous examples of enzymes created by the disclosed methods, such enzymes providing the claimed improved background specificity. As such, Applicants assert that the present application clearly satisfies the requirements of 35 U.S.C. §112, first paragraph cited by the Examiner as his basis for this rejection. Applicants respectfully

request that this rejection be withdrawn.

CONCLUSION

For the reasons set forth above, it is respectfully submitted that Applicants' claims should be passed to allowance. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicants encourages the Examiner to call the undersigned collect at (608) 218-6900.

Dated: December 9, 2002

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